Protocol

Protocol for LB medium preparation（1L）	
1.Ingredients:	
Tryptone	10mL
Yeast extract	10mL
NaCl	10mL
Deionized water	1mL
Agar	1.8%（18g）——if needed
2.High pressure of steam sterilization(121℃, 15 min)	
Add 1000μL of Ampicillin——if needed	

Protocol for TAP medium preparation（1L）	
1.Ingredients:	
100×phosphate buffer	10mL
100×Beijerinck's solution	10mL
100×Tris-Acetate	10mL
1000×Trace element	1mL
Agar	1.8%（18g）——if needed
3.High pressure of steam sterilization(121℃, 15 min)	
Add 1000μL of Ampicillin,Paromomycin and Hygromycin B——if needed	

Protocol for PCR

①Phanta Max Super-Fidelity DNA Polymerase

(1)Prepare the reaction mix in PCR tube:

2×Phanta Max Buffer	25μL
dNTP Mix	1μL
Template DNA	to 30ng
Forward primer	1μL
Reverse primer	1μL
Phanta Max Super-Fidelity DNA Polymerase	1μL
ddH_2O	to 50μL

（2）Set the program of thermal cycler:

95℃	30s	

95℃	15s	35 cycles
TM	15s	35 cycles
72℃	1000bp/min	35 cycles
72℃	5min	
4℃	+ ∞	

②2×Easy Taq Super Mix

(1) Prepare the reaction mix in PCR tube:

2×EasyTaq PCR SuperMix	25μL
Template DNA	to 30ng
Forward primer	1μL
Reverse primer	1μL
ddH_2O	to 50μL

(2) Set the program of thermal cycler:

94℃	5min	
94℃	30s	35cycles
TM	30s	35cycles
72℃	1000bp/min	35cycles
72℃	5min	
4℃	+ ∞	

Protocol for connection to T-Vector

1. Prepare reaction mix according to the following table

PCR product	4μL
Peasy-T1 Simple Cloning Vector	1μL

2.incubate at 25℃ for 5min

Protocol for transformation to competent cells

1. Take the competent cells out of the -80℃ freezer.
2. Put the competent cells in the ice for 5 minutes.
3. Add 5ul of the ligation product to the competent cells and put them in the ice for 20-30 minutes.
4. Next put the competent cells in water about 42℃ for 45 seconds. Then put the competent cells in ice for 2 minutes immediately.
5. Add 250μl LB liquid medium（without Ampicillin resistance） to the competent cells and put them into the Incubator shaker for 1 hour（37℃ 200rpm）
6. Take 250μl of the competent cells into the LB plate containing corresponding antibiotics and spread them on the plate evenly.
7. Put the LB plate to the Incubator for 14-16 hours.

Protocol for plasmids extraction

1.Add 500µl Buffer BL to spin column CP3. Centrifuge for 1 min at 12,000 rpm in a table-top microcentrifuge. Discard the flow-throw, and place Spin Column CP3 into the collection tube.

2.Harvest 1.4 ml bacterial cells in a microcentifuge tube by centrifuge for 1 min at 12,000 rpm for 1 min at 20℃, then remove all the traces of supernatant. Then redo with 1.4ml bacterial cells in another microcentifuge tube.

3.Resuspend pelleted bacterial cells in 250µl Buffer P1

4.Add 250µl Buffer P2 and mix thoroughly by inverting the tube 6-8 times

5.Add 350µl Buffer P3 and mix immediately and thoroughly by inverting the tube 6-8 times

6.Centrifuge for 10min at 12,000 rpm

7.Apply the supernatants to the Spin Column CP3, centrifuge for 1min at 12,000 rpm 8.Wash the Spin Column CP3 by adding 700µl Buffer PW and centrifuge for 1min at 12,000 rpm. Discard the flow-through, wash again with 500µl Buffer PW and centrifuge for 1min at 12,000 rpm.

9. Discard the flow-through and centrifuge for 2min at 12,000 rpm

10.Place the Spin Column CP3 in a clean 1.5ml microcentrifuge tube. Add 50µl EB, let stand for 4min, and centrifuge for 2 min at 12,000 rpm.

Protocol for Enzyme digestion

1. Prepare reaction mix according to the following table

Fragment or plasmid need to digest	16µL
corresponding enzymes of upstream restriction Enzyme cutting site	1µL
corresponding enzymes of downstream restriction Enzyme cutting site	1µL
Enzyme buffer (Buffer)	2µL

2. incubate at recommended temperature (37℃) for at least 1 hour.

3. Purify the digestion product by inactivating at 80 ° C.

Protocol for DNA Ligation

1. Prepare reaction mix according to the following table

digested fragment/vector	8.5µL
T4 DNA ligase	0.5µL
10x T4 DNA ligase buffer	1µL

2. Ligate the mix overnight at 16℃

Protocol for glass bead method（transformation to Chlamydomonas reinhardtii）

Nuclear transformation of C. reinhardtii. All transformations were carried out using the glass-bead method of Kindle[8J •

For transformation:

(i) the cells were grown to mid-log phase (1-2 X 106 cells/ mL) in TAP medium, then pelleted at 23 'C, and resuspended in fresh TAP to a concentration of 2 X 108cells/ mL.

(ii) 300 flL of the cell suspension was transferred to 5 mL test tubes containing 0.3 g of 0.4 mm diameter glass beads. 1 flg of linearised DNA was added and the celli glass bead! DNA suspension vortexed for 15 s at top speed.
(iii) cells were transferred to a 25 mL tube containing 10 mL of TAP medium, and incubated overnight.
(iv) cells were pelleted by centrifugation at 23'C, and resuspended gently in 0.5 mL TAP medium. It was added with 3.5 mL of TAP-0.5% agar and poured onto TAP-2% agar supplemented with 10 flg/mL Zeomycin, and finally incubated in the light (45 flE/m2 /s) at 22 'C. Transformants are visible after 3-4 weeks

Protocol for DNA extraction for green algae

Before I centrifuge the cells, I filter the cells through a 3um filter and wash with either JM media or sterile MQ water..
DNA Extraction Protocol (Filamentous Cyanobacteria)

1. Centrifuge around 5ml culture (100mg) cells into pellet
2. Resuspend in 0.5ml of Buffer A (0.15M Nacl and 0.1M EDTA) in 2ml cryovials or 2ml eppendorf tubes
3. Perform 3 freeze thaws between liquid Nitrogen and 37℃
4. Centrifuge for 10mins at 8000rpm and remove supernatant
5. Resuspend in 0.5ml TE buffer and transfer to 2ml microcentrifuge tubes
6. Add 100ul (50mg/ml) lysozyme + incubate for 30mins at 37℃
7. Add 5ul (50mg/ml) proteinase K + SDS to a final concentration of 2%. Incubate at 55℃ for 1 hour. (600ul volume/ 20%SDS, 60ul)
8. Add 150ul 5M Nacl and 0.1volume 10%CTAB
9. Mix by inversion and incubate at 65℃ for 10mins
10. Add 1 volume chloroform. Place on ice for 30mins. Then centrifuge at 8000rpm for 10 mins
11. Pipette out the DNA in the top layer, add one volume phenol, then centrifuge at 8000rpm for 10 mins
12. Pipette out the DNA top layer, add 2 volumes of 100% ethanol. Leave overnight at 4℃ 13. Centrifuge 10,000rpm for 30mins
14. Wash with 70% ethanol
15. Centrifuge 10,000rpm for 15 mins
16. Leave to dry, then resuspend in TE.